METHODS OF ENHANCING AND/OR STABILIZING CARDIAC FUNCTION IN PATIENTS WITH FABRY DISEASE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Serial No. 62/550,984 filed on August 28, 2017, which is incorporated by reference herein in its entirety.

TECHNICAL FIELD

[0002] Principles and embodiments of the present invention relate generally to the use of pharmacological chaperones for the treatment of lysosomal storage disorders, particularly the use of migalastat for the treatment of Fabry disease.

BACKGROUND

[0003] Fabry disease is a progressive, X-linked inborn error of glycosphingolipid metabolism caused by a deficiency in the lysosomal enzyme α-galactosidase A (α-Gal A) as a result of mutations in the α-Gal A gene (GLA). Despite being an X-linked disorder, females can express varying degrees of clinical manifestations. Fabry is a rare disease with incidence estimated between 1 in 40,000 males to 1 in 117,000 in the general population. Moreover, there are variants of later onset phenotype of Fabry disease that can be under-diagnosed, as they do not present with classical signs and symptoms. This, and newborn screening for Fabry disease, suggests that the actual incidence of Fabry disease can be higher than currently estimated.

[0004] Untreated, life expectancy in Fabry patients is reduced and death usually occurs in the fourth or fifth decade because of vascular disease affecting the kidneys, heart and/or central nervous system. The enzyme deficiency leads to intracellular accumulation of the substrate, globotriaosylceramide (GL-3) in the vascular endothelium and visceral tissues throughout the body. Gradual deterioration of renal function and the development of azotemia, due to glycosphingolipid deposition, usually occur in the third to fifth decades of life, but can occur as early as in the second decade. Renal lesions are found in both hemizygous (male) and heterozygous (female) patients.

[0005] Cardiac disease as a result of Fabry disease occurs in most males and many females. Early cardiac findings include left ventricular enlargement, valvular involvement and

conduction abnormalities. Mitral insufficiency is the most frequent valvular lesion typically present in childhood or adolescence. Cerebrovascular manifestations result primarily from multifocal small-vessel involvement and can include thromboses, transient ischemic attacks, basilar artery ischemia and aneurysm, seizures, hemiplegia, hemianesthesia, aphasia, labyrinthine disorders, or cerebral hemorrhages. Average age of onset of cerebrovascular manifestations is 33.8 years. Personality change and psychotic behavior can manifest with increasing age.

[0006] One approved therapy for treating Fabry disease is enzyme replacement therapy (ERT), which typically involves intravenous, infusion of a purified form of the corresponding wild-type protein. Two α-Gal A products are currently available for the treatment of Fabry disease: agalsidase alfa (Replagal®, Shire Human Genetic Therapies) and agalsidase beta (Fabrazyme®; Sanofi Genzyme Corporation). While ERT is effective in many settings, the treatment also has limitations. ERT has not been demonstrated to decrease the risk of stroke, cardiac muscle responds slowly, and GL-3 elimination from some of the cell types of the kidneys is limited. Some patients also develop immune reactions to ERT.

[0007] Accordingly, there remains a need for therapies for the treatment of Fabry disease, especially for enhancing the cardiac function.

SUMMARY

[0008] Various aspects of the present invention relate to the treatment of Fabry disease in ERT-naïve and ERT-experienced patients using migalastat. Such treatment can include enhancing and/or stabilizing cardiac function, such as increasing and/or stabilizing midwall fractional shortening (MWFS).

[0009] One aspect of the present invention pertains to a method of enhancing cardiac function in a patient having Fabry disease, the method comprising administering to the patient a formulation comprising an effective amount of migalastat or salt thereof every other day for enhancing the patient's cardiac function, wherein the effective amount is about 100 mg to about 150 mg free base equivalent (FBE).

[0010] In one or more embodiments, enhancing cardiac function comprises enhancing left ventricular systolic function.

[0011] In one or more embodiments, the patient has impaired MWFS prior to initiating administration of the migalastat or salt thereof.

[0012] In one or more embodiments, the patient has left ventricular hypertrophy (LVH) prior to initiating administration of the migalastat or salt thereof.

[0013] In one or more embodiments, the migalastat or salt thereof enhances α-Gal A activity.

[0014] In one or more embodiments, the patient is administered about 123 mg FBE of the migalastat or salt thereof every other day.

[0015] In one or more embodiments, the patient is administered about 123 mg of migalastat free base every other day.

[0016] In one or more embodiments, the patient is administered about 150 mg of migalastat hydrochloride every other day.

[0017] In one or more embodiments, the formulation comprises an oral dosage form. In one or more embodiments, the oral dosage form comprises a tablet, a capsule or a solution.

[0018] In one or more embodiments, the migalastat or salt thereof is administered for at least 12 months.

[0019] In one or more embodiments, the migalastat or salt thereof is administered for at least 24 months.

[0020] In one or more embodiments, the patient is an ERT-naïve patient.

[0021] In one or more embodiments, the administration of migalastat or a salt thereof provides an average increase in MWFS in a group of ERT-naïve patients with impaired MWFS of at least about 1% after 24 months of administration of migalastat or a salt thereof.

[0022] In one or more embodiments, the patient is an ERT-experienced patient.

[0023] Another aspect of the present invention pertains to a method of increasing MWFS in a patient having Fabry disease, the method comprising administering to the patient a formulation comprising an effective amount of migalastat or salt thereof every other day for increasing the patient's MWFS, wherein the effective amount is about 100 mg to about 150 mg FBE.

[0024] In one or more embodiments, the patient has impaired MWFS prior to initiating administration of the migalastat or salt thereof.

[0025] In one or more embodiments, the patient has LVH prior to initiating administration of the migalastat or salt thereof.

[0026] In one or more embodiments, the migalastat or salt thereof enhances α-Gal A activity.

[0027] In one or more embodiments, the patient is administered about 123 mg FBE of the migalastat or salt thereof every other day.

[0028] In one or more embodiments, the patient is administered about 123 mg of migalastat free base every other day.

[0029] In one or more embodiments, the patient is administered about 150 mg of migalastat hydrochloride every other day.

[0030] In one or more embodiments, the formulation comprises an oral dosage form. In one or more embodiments, the oral dosage form comprises a tablet, a capsule or a solution.

[0031] In one or more embodiments, the migalastat or salt thereof is administered for at least 12 months.

[0032] In one or more embodiments, the migalastat or salt thereof is administered for at least 24 months.

[0033] In one or more embodiments, the patient is an ERT-naïve patient.

[0034] In one or more embodiments, the administration of migalastat or a salt thereof provides an average increase in MWFS in a group of ERT-naïve patients with impaired MWFS of at least about 1% after 24 months of administration of migalastat or a salt thereof.

[0035] In one or more embodiments, the patient is an ERT-experienced patient.

[0036] Another aspect of the present invention pertains to a method of normalizing MWFS in a patient having Fabry disease and impaired MWFS, the method comprising administering to the patient a formulation comprising an effective amount of migalastat or salt thereof every other day for normalizing the patient's MWFS, wherein the effective amount is about 100 mg to about 150 mg FBE.

[0037] In one or more embodiments, the migalastat or salt thereof enhances α-Gal A activity.

[0038] In one or more embodiments, the patient is administered about 123 mg FBE of the migalastat or salt thereof every other day.

[0039] In one or more embodiments, the patient is administered about 123 mg of migalastat free base every other day.

[0040] In one or more embodiments, the patient is administered about 150 mg of migalastat hydrochloride every other day.

[0041] In one or more embodiments, the formulation comprises an oral dosage form. In one or more embodiments, the oral dosage form comprises a tablet, a capsule or a solution.

[0042] In one or more embodiments, the migalastat or salt thereof is administered for at least 12 months.

[0043] In one or more embodiments, the migalastat or salt thereof is administered for at least 24 months.

[0044] In one or more embodiments, the patient is an ERT-naïve patient.

[0045] In one or more embodiments, the administration of migalastat or a salt thereof provides an average increase in MWFS in a group of ERT-naïve patients with impaired MWFS of at least about 1% after 24 months of administration of migalastat or a salt thereof.

[0046] Another aspect of the present invention pertains to a method of stabilizing MWFS in a patient having Fabry disease, the method comprising administering to the patient a formulation comprising an effective amount of migalastat or salt thereof every other day for stabilizing the patient's MWFS, wherein the effective amount is about 100 mg to about 150 mg FBE.

[0047] In one or more embodiments, the patient has impaired MWFS prior to initiating administration of the migalastat or salt thereof.

[0048] In one or more embodiments, the patient has LVH prior to initiating administration of the migalastat or salt thereof.

[0049] In one or more embodiments, the migalastat or salt thereof enhances α-Gal A activity.

[0050] In one or more embodiments, the patient is administered about 123 mg FBE of the migalastat or salt thereof every other day.

[0051] In one or more embodiments, the patient is administered about 123 mg of migalastat free base every other day.

[0052] In one or more embodiments, the patient is administered about 150 mg of migalastat hydrochloride every other day.

[0053] In one or more embodiments, the formulation comprises an oral dosage form. In one or more embodiments, the oral dosage form comprises a tablet, a capsule or a solution.

[0054] In one or more embodiments, the migalastat or salt thereof is administered for at least 12 months.

[0055] In one or more embodiments, the migalastat or salt thereof is administered for at least 30 months.

[0056] In one or more embodiments, the patient is an ERT-experienced patient.

[0057] In one or more embodiments, the administration of migalastat or a salt thereof provides an average change in MWFS in a group of ERT-experienced patients with impaired MWFS of greater than about -0.5% after 30 months of administration of migalastat or a salt thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0058] Further features of the present invention will become apparent from the following written description and the accompanying figures, in which:

[0059] FIGS. 1A-E show the full DNA sequence of the human wild-type GLA gene (SEQ ID NO: 1);

[0060] FIG. 2 shows the wild-type α-Gal A protein (SEQ ID NO: 2); and

[0061] FIG. 3 shows the nucleic acid sequence encoding the wild-type α-Gal A protein (SEQ ID NO: 3).

DETAILED DESCRIPTION

[0062] Before describing several exemplary embodiments of the invention, it is to be understood that the invention is not limited to the details of construction or process steps set forth in the following description. The invention is capable of other embodiments and of being practiced or being carried out in various ways.

[0063] Various aspects of the present invention pertain to dosing regimens for the administration of pharmacological chaperones such as migalastat for the treatment of Fabry disease. In one or more embodiments, the dosing regimens of migalastat enhance one or more cardiac parameters of a patient.

Definitions

[0064] The terms used in this specification generally have their ordinary meanings in the art, within the context of this invention and in the specific context where each term is used. Certain terms are discussed below, or elsewhere in the specification, to provide additional guidance to the practitioner in describing the compositions and methods of the invention and how to make and use them.

[0065] The term "Fabry disease" refers to an X-linked inborn error of glycosphingolipid catabolism due to deficient lysosomal α-Gal A activity. This defect causes accumulation of the

substrate globotriaosylceramide ("GL-3", also known as Gb3 or ceramide trihexoside) and related glycosphingolipids in vascular endothelial lysosomes of the heart, kidneys, skin, and other tissues. Another substrate of the enzyme is plasma globotriaosylsphingosine ("plasma lyso-Gb_3").

[0066] The term "atypical Fabry disease" refers to patients with primarily cardiac manifestations of the α-Gal A deficiency, namely progressive GL-3 accumulation in myocardial cells that leads to significant enlargement of the heart, particularly the left ventricle.

[0067] A "carrier" is a female who has one X chromosome with a defective α-Gal A gene and one X chromosome with the normal gene and in whom X chromosome inactivation of the normal allele is present in one or more cell types. A carrier is often diagnosed with Fabry disease.

[0068] A "patient" refers to a subject who has been diagnosed with or is suspected of having a particular disease. The patient may be human or animal.

[0069] A "Fabry patient" refers to an individual who has been diagnosed with or suspected of having Fabry disease and has a mutated α-Gal A as defined further below. Characteristic markers of Fabry disease can occur in male hemizygotes and female carriers with the same prevalence, although females typically are less severely affected.

[0070] Human α-galactosidase A (α-Gal A) refers to an enzyme encoded by the human GLA gene. The full DNA sequence of α-Gal A, including introns and exons, is available in GenBank Accession No. X14448.1 and shown in FIG. 1A-E (SEQ ID NO: 1). The human α-Gal A enzyme consists of 429 amino acids and is available in GenBank Accession Nos. X14448.1 and U78027.1 and shown in FIG.2 (SEQ ID NO: 2). The nucleic acid sequence that only includes the coding regions (i.e. exons) of SEQ ID NO: 1 is shown in FIG. 3 (SEQ ID NO: 3).

[0071] The term "mutant protein" includes a protein which has a mutation in the gene encoding the protein which results in the inability of the protein to achieve a stable conformation under the conditions normally present in the endoplasmic reticulum (ER). The failure to achieve a stable conformation results in a substantial amount of the enzyme being degraded, rather than being transported to the lysosome. Such a mutation is sometimes called a "conformational mutant." Such mutations include, but are not limited to, missense mutations, and in-frame small deletions and insertions.

[0072] As used herein in one embodiment, the term "mutant α-Gal A" includes an α-Gal A which has a mutation in the gene encoding α-Gal A which results in the inability of the enzyme to achieve a stable conformation under the conditions normally present in the ER. The failure to achieve a stable conformation results in a substantial amount of the enzyme being degraded, rather than being transported to the lysosome.

[0073] As used herein, the term "pharmacological chaperone" ("PC") refers to any molecule including a small molecule, protein, peptide, nucleic acid, carbohydrate, etc. that specifically binds to a protein and has one or more of the following effects: (i) enhances the formation of a stable molecular conformation of the protein; (ii) induces trafficking of the protein from the ER to another cellular location, preferably a native cellular location, *i.e.*, prevents ER-associated degradation of the protein; (iii) prevents aggregation of misfolded proteins; and/or (iv) restores or enhances at least partial wild-type function and/or activity to the protein. A compound that specifically binds to *e.g.*, α-Gal A, means that it binds to and exerts a chaperone effect on the enzyme and not a generic group of related or unrelated enzymes. More specifically, this term does not refer to endogenous chaperones, such as BiP, or to non-specific agents which have demonstrated non-specific chaperone activity against various proteins, such as glycerol, DMSO or deuterated water, *i.e.*, chemical chaperones. In one or more embodiments of the present invention, the PC may be a reversible competitive inhibitor. In one embodiment, the PC is migalastat or a salt thereof. In another embodiment, the PC is migalastat free base (*e.g.*, 123 mg of migalastat free base). In yet another embodiment, the PC is a salt of migalastat (*e.g.*, 150 mg of migalastat HCl).

[0074] A "competitive inhibitor" of an enzyme can refer to a compound which structurally resembles the chemical structure and molecular geometry of the enzyme substrate to bind the enzyme in approximately the same location as the substrate. Thus, the inhibitor competes for the same active site as the substrate molecule, thus increasing the Km. Competitive inhibition is usually reversible if sufficient substrate molecules are available to displace the inhibitor, *i.e.*, competitive inhibitors can bind reversibly. Therefore, the amount of enzyme inhibition depends upon the inhibitor concentration, substrate concentration, and the relative affinities of the inhibitor and substrate for the active site.

[0075] As used herein, the term "specifically binds" refers to the interaction of a pharmacological chaperone with a protein such as α-Gal A, specifically, an interaction with amino acid residues of the protein that directly participate in contacting the pharmacological

chaperone. A pharmacological chaperone specifically binds a target protein, *e.g.*, α-Gal A, to exert a chaperone effect on the protein and not a generic group of related or unrelated proteins. The amino acid residues of a protein that interact with any given pharmacological chaperone may or may not be within the protein's "active site." Specific binding can be evaluated through routine binding assays or through structural studies, *e.g.*, co-crystallization, NMR, and the like. The active site for α-Gal A is the substrate binding site.

[0076] "Deficient α-Gal A activity" refers to α-Gal A activity in cells from a patient which is below the normal range as compared (using the same methods) to the activity in normal individuals not having or suspected of having Fabry or any other disease (especially a blood disease).

[0077] As used herein, the terms "enhance α-Gal A activity" or "increase α-Gal A activity" refer to increasing the amount of α-Gal A that adopts a stable conformation in a cell contacted with a pharmacological chaperone specific for the α-Gal A, relative to the amount in a cell (preferably of the same cell-type or the same cell, *e.g.*, at an earlier time) not contacted with the pharmacological chaperone specific for the α-Gal A. This term also refers to increasing the trafficking of α-Gal A to the lysosome in a cell contacted with a pharmacological chaperone specific for the α-Gal A, relative to the trafficking of α-Gal A not contacted with the pharmacological chaperone specific for the protein. These terms refer to both wild-type and mutant α-Gal A. In one embodiment, the increase in the amount of α-Gal A in the cell is measured by measuring the hydrolysis of an artificial substrate in lysates from cells that have been treated with the PC. An increase in hydrolysis is indicative of increased α-Gal A activity.

[0078] The term "α-Gal A activity" refers to the normal physiological function of a wild-type α-Gal A in a cell. For example, α-Gal A activity includes hydrolysis of GL-3.

[0079] A "responder" is an individual diagnosed with or suspected of having a lysosomal storage disorder (LSD), such, for example Fabry disease, whose cells exhibit sufficiently increased α-Gal A activity, respectively, and/or amelioration of symptoms or enhancement in surrogate markers, in response to contact with a PC. Non-limiting examples of enhancements in surrogate markers for Fabry are lyso-GB3 and those disclosed in US Patent Application Publication No. U.S. 2010/0113517, which is hereby incorporated by reference in its entirety.

[0080] Non-limiting examples of improvements in surrogate markers for Fabry disease disclosed in U.S. 2010/0113517 include increases in α-Gal A levels or activity in cells (*e.g.*, fibroblasts) and tissue; reductions in of GL-3 accumulation; decreased plasma concentrations of homocysteine and vascular cell adhesion molecule-1 (VCAM-1); decreased GL-3 accumulation within myocardial cells and valvular fibrocytes; reduction in plasma lyso-Gb_3; reduction in cardiac hypertrophy (especially of the left ventricle), amelioration of valvular insufficiency, and arrhythmias; amelioration of proteinuria; decreased urinary concentrations of lipids such as CTH, lactosylceramide, ceramide, and increased urinary concentrations of glucosylceramide and sphingomyelin; the absence of laminated inclusion bodies (Zebra bodies) in glomerular epithelial cells; improvements in renal function; mitigation of hypohidrosis; the absence of angiokeratomas; and improvements in hearing abnormalities such as high frequency sensorineural hearing loss progressive hearing loss, sudden deafness, or tinnitus. Improvements in neurological symptoms include prevention of transient ischemic attack (TIA) or stroke; and amelioration of neuropathic pain manifesting itself as acroparaesthesia (burning or tingling in extremities). Another type of clinical marker that can be assessed for Fabry disease is the prevalence of deleterious cardiovascular manifestations.

[0081] "Midwall fractional shortening" or "MWFS" is a measure of systolic function that identifies hypertensive patients who have evidence of target-organ damage, impaired contractile reserve, and increased mortality.

[0082] The term "cardiac function" refers to the performance of a patient's heart. For example, one assessment of cardiac function is left ventricular systolic function, which refers to the emptying characteristics of the left heart. Left ventricular systolic function can be evaluated in several ways, including, but not limited to, left ventricular ejection fraction (LVEF), endocardial fractional shortening (EFS) and MWFS.

[0083] As used herein, the phrase "stabilizing cardiac function" and similar terms refer to reducing or arresting the decline in cardiac function and/or restoring cardiac function. As untreated Fabry patients are expected to have significant decreases in cardiac function over time, enhancements in the rate of cardiac function deterioration and/or enhancements in cardiac function demonstrate a benefit of migalastat therapy as described herein. In various embodiments, stabilizing cardiac function includes stabilizing MWFS. "Stabilizing MWFS" likewise refers to reducing or arresting the decline in MWFS.

[0084] The term "enhancing cardiac function" refers to a beneficial change in at least one parameter used to evaluate cardiac function. If a patient's parameter is at the lower end of a normal range or is below the normal range for that parameter, than a beneficial change in that parameter is an increase in that parameter. For example, an increase in MWFS for a patient having a low MWFS is an enhancement in that parameter. Similarly, if a patient's parameter is at the upper end of a normal range or is above the normal range for that parameter, than a beneficial change in that parameter is a decrease in that parameter. In an aspect, "enhancing cardiac function" comprises one or more of (i) improving left ventricular function, (ii) improving fractional shortening, (iii) improving ejection fraction, (iv) reducing end-diastolic volume, and (v) normalizing of heart geometry.

[0085] As used herein, the term "impaired MWFS" refers to a patient having an MWFS below the normal range. The normal range of MWFS for a female is at least 15% and the normal range of MWFS for a male is at least 14%. Thus, impaired MWFS for a female patient is <15% and impaired MWFS for a male patient is <14%.

[0086] As used herein, the term "normalizing MWFS" refers to increasing the MWFS of a patient from an impaired MWFS to within the normal range. Thus, normalizing MWFS for a female patient is increasing MWFS from <15% to at least 15%, and normalizing MWFS for a male patient is increasing MWFS from <14% to at least 14%.

[0087] As used herein, the term "left ventricular hypertrophy" or "LVH" refers to a patient having a left ventricular mass index (LVMi) above the normal range of 43-95 g/m^2 for females and 49-115 g/m^2 for males. Thus, LVH refers to a LVMi > 95 g/m^2 for females or > 115 g/m^2 for males.

[0088] The dose that achieves one or more of the aforementioned responses is a "therapeutically effective dose."

[0089] The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce untoward reactions when administered to a human. In some embodiments, as used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals, and more particularly in humans. The term "carrier" in reference to a pharmaceutical carrier refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water

and oils. Water or aqueous solution saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin, 18th Edition, or other editions.

[0090] As used herein, the term "isolated" means that the referenced material is removed from the environment in which it is normally found. Thus, an isolated biological material can be free of cellular components, *i.e.*, components of the cells in which the material is found or produced. In the case of nucleic acid molecules, an isolated nucleic acid includes a PCR product, an mRNA band on a gel, a cDNA, or a restriction fragment. In another embodiment, an isolated nucleic acid is preferably excised from the chromosome in which it may be found, and more preferably is no longer joined to non-regulatory, non-coding regions, or to other genes, located upstream or downstream of the gene contained by the isolated nucleic acid molecule when found in the chromosome. In yet another embodiment, the isolated nucleic acid lacks one or more introns. Isolated nucleic acids include sequences inserted into plasmids, cosmids, artificial chromosomes, and the like. Thus, in a specific embodiment, a recombinant nucleic acid is an isolated nucleic acid. An isolated protein may be associated with other proteins or nucleic acids, or both, with which it associates in the cell, or with cellular membranes if it is a membrane-associated protein. An isolated organelle, cell, or tissue is removed from the anatomical site in which it is found in an organism. An isolated material may be, but need not be, purified.

[0091] The term "enzyme replacement therapy" or "ERT" refers to the introduction of a non-native, purified enzyme into an individual having a deficiency in such enzyme. The administered protein can be obtained from natural sources or by recombinant expression (as described in greater detail below). The term also refers to the introduction of a purified enzyme in an individual otherwise requiring or benefiting from administration of a purified enzyme, *e.g.*, suffering from enzyme insufficiency. The introduced enzyme may be a purified, recombinant enzyme produced *in vitro*, or protein purified from isolated tissue or fluid, such as, *e.g.*, placenta or animal milk, or from plants.

[0092] The term "ERT-naïve patient" refers to a Fabry patient that has never received ERT or has not received ERT for at least 6 months prior to initiating migalastat therapy.

[0093] The term "ERT-experienced patient" refers to a Fabry patient that was receiving ERT immediately prior to initiating migalastat therapy. In some embodiments, the ERT-

experienced patient has received at least 12 months of ERT immediately prior to initiating migalastat therapy.

[0094] As used herein, the term "free base equivalent" or "FBE" refers to the amount of migalastat present in the migalastat or salt thereof. In other words, the term "FBE" means either an amount of migalastat free base, or the equivalent amount of migalastat free base that is provided by a salt of migalastat. For example, due to the weight of the hydrochloride salt, 150 mg of migalastat hydrochloride only provides as much migalastat as 123 mg of the free base form of migalastat. Other salts are expected to have different conversion factors, depending on the molecular weight of the salt.

[0095] The term "migalastat" encompasses migalastat free base or a pharmaceutically acceptable salt thereof (*e.g.*, migalastat HCl), unless specifically indicated to the contrary.

[0096] The terms "mutation" and "variant" (*e.g.*, as in "amenable mutation or variant") refer to a change in the nucleotide sequence of a gene or a chromosome. The two terms referred herein are typically used together – *e.g.*, as in "mutation or variant"– referring to the change in nucleotide sequence stated in the previous sentence. If only one of the two terms is recited for some reason, the missing term was intended to be included and one should understand as such. Furthermore, the terms "amenable mutation" and "amenable variant" refer to a mutation or variant that is amenable to PC therapy, *e*.g., a mutation that is amenable to migalastat therapy. A particular type of amenable mutation or variant is a "HEK assay amenable mutation or variant", which is a mutation or variant that is determined to be amenable to migalastat therapy according to the criteria in the *in vitro* HEK assay described herein and in U.S. Patent No. 8,592,362, which is hereby incorporated by reference in its entirety.

[0097] The terms "about" and "approximately" shall generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Typical, exemplary degrees of error are within 20 percent (%), preferably within 10%, and more preferably within 5% of a given value or range of values. Alternatively, and particularly in biological systems, the terms "about" and "approximately" may mean values that are within an order of magnitude, preferably within 10- or 5-fold, and more preferably within 2-fold of a given value. Numerical quantities given herein are approximate unless stated otherwise, meaning that the term "about" or "approximately" can be inferred when not expressly stated.

Fabry Disease

[0098] Fabry disease is a rare, progressive and devastating X-linked LSD. Mutations in the GLA gene result in a deficiency of the lysosomal enzyme, α-Gal A, which is required for glycosphingolipid metabolism. Beginning early in life, the reduction in α-Gal A activity results in an accumulation of glycosphingolipids, including GL-3 and plasma lyso-Gb3, and leads to the symptoms and life-limiting sequelae of Fabry disease, including pain, gastrointestinal symptoms, renal failure, cardiomyopathy, cerebrovascular events, and early mortality. Early initiation of therapy and lifelong treatment provide an opportunity to slow disease progression and prolong life expectancy.

[0099] Fabry disease encompasses a spectrum of disease severity and age of onset, although it has traditionally been divided into 2 main phenotypes, "classic" and "late-onset". The classic phenotype has been ascribed primarily to males with undetectable to low α-Gal A activity and earlier onset of renal, cardiac and/or cerebrovascular manifestations. The late-onset phenotype has been ascribed primarily to males with higher residual α-Gal A activity and later onset of these disease manifestations. Heterozygous female carriers typically express the late-onset phenotype but depending on the pattern of X-chromosome inactivation may also display the classic phenotype.

[00100] More than 1,000 Fabry disease-causing GLA mutations have been identified. Approximately 60% are missense mutations, resulting in single amino acid substitutions in the α-Gal A enzyme. Missense GLA mutations often result in the production of abnormally folded and unstable forms of α-Gal A and the majority are associated with the classic phenotype. Normal cellular quality control mechanisms in the ER block the transit of these abnormal proteins to lysosomes and target them for premature degradation and elimination. Many missense mutant forms are targets for migalastat, an α-Gal A-specific pharmacological chaperone.

[00101] The clinical manifestations of Fabry disease span a broad spectrum of severity and roughly correlate with a patient's residual α-Gal A levels. The majority of currently treated patients are referred to as classic Fabry patients, most of whom are males. These patients experience disease of various organs, including the kidneys, heart and brain, with disease symptoms first appearing in adolescence and typically progressing in severity until death in the fourth or fifth decade of life. A number of recent studies suggest that there are a large number of undiagnosed males and females that have a range of Fabry disease symptoms, such as

impaired cardiac or renal function and strokes, that usually first appear in adulthood. Individuals with this type of Fabry disease, referred to as later-onset Fabry disease, tend to have higher residual α-Gal A levels than classic Fabry patients. Individuals with later-onset Fabry disease typically first experience disease symptoms in adulthood, and often have disease symptoms focused on a single organ, such as enlargement of the left ventricle or progressive kidney failure. In addition, later-onset Fabry disease may also present in the form of strokes of unknown cause.

[00102] Fabry patients have progressive kidney impairment, and untreated patients exhibit end-stage renal impairment by the fifth decade of life. Deficiency in α-Gal A activity leads to accumulation of GL-3 and related glycosphingolipids in many cell types including cells in the kidney. GL-3 accumulates in podocytes, epithelial cells and the tubular cells of the distal tubule and loop of Henle. Impairment in kidney function can manifest as proteinuria and reduced glomerular filtration rate.

[00103] Because Fabry disease is rare, involves multiple organs, has a wide age range of onset, and is heterogeneous, proper diagnosis is a challenge. Awareness is low among health care professionals and misdiagnoses are frequent. Diagnosis of Fabry disease is most often confirmed on the basis of decreased α-Gal A activity in plasma or peripheral leukocytes (WBCs) once a patient is symptomatic, coupled with mutational analysis. In females, diagnosis is even more challenging since the enzymatic identification of carrier females is less reliable due to random X-chromosomal inactivation in some cells of carriers. For example, some obligate carriers (daughters of classically affected males) have α-Gal A enzyme activities ranging from normal to very low activities. Since carriers can have normal α-Gal A enzyme activity in leukocytes, only the identification of an α-Gal A mutation by genetic testing provides precise carrier identification and/or diagnosis.

[00104] In one or more embodiments, mutant forms of α-Gal A are considered to be amenable to migalastat are defined as showing a relative increase (+10 μM migalastat) of ≥1.20-fold and an absolute increase (+ 10 μM migalastat) of ≥ 3.0% wild-type (WT) when the mutant form of α-Gal A is expressed in HEK-293 cells (referred to as the "HEK assay") according to Good Laboratory Practice (GLP)-validated *in vitro* assay (GLP HEK or Migalastat Amenability Assay). Such mutations are also referred to herein as "HEK assay amenable" mutations.

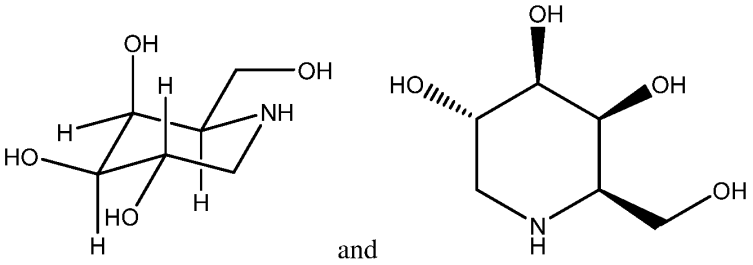
[00105] Previous screening methods have been provided that assess enzyme enhancement prior to the initiation of treatment. For example, an assay using HEK-293 cells has been utilized in clinical trials to predict whether a given mutation will be responsive to pharmacological chaperone (*e.g.*, migalastat) treatment. In this assay, cDNA constructs are created. The corresponding α-Gal A mutant forms are transiently expressed in HEK-293 cells. Cells are then incubated ± migalastat (17 nM to 1 mM) for 4 to 5 days. After, α-Gal A levels are measured in cell lysates using a synthetic fluorogenic substrate (4-MU-α-Gal) or by western blot. This has been done for known disease-causing missense or small in-frame insertion/deletion mutations. Mutations that have previously been identified as responsive to a PC (*e.g.*, migalastat) using these methods are listed in U.S. Patent No. 8,592,362.

Pharmacological Chaperones

[00106] The binding of small molecule inhibitors of enzymes associated with LSDs can increase the stability of both mutant enzyme and the corresponding wild-type enzyme (see U.S. Pat. Nos. 6,274,597; 6,583,158; 6,589,964; 6,599,919; 6,916,829, and 7,141,582 all incorporated herein by reference). In particular, administration of small molecule derivatives of glucose and galactose, which are specific, selective competitive inhibitors for several target lysosomal enzymes, effectively increased the stability of the enzymes in cells *in vitro* and, thus, increased trafficking of the enzymes to the lysosome. Thus, by increasing the amount of enzyme in the lysosome, hydrolysis of the enzyme substrates is expected to increase. The original theory behind this strategy was as follows: since the mutant enzyme protein is unstable in the ER (Ishii *et al.*, *Biochem. Biophys. Res. Comm.* 1996; 220: 812-815), the enzyme protein is retarded in the normal transport pathway (ER→Golgi apparatus→endosomes→lysosome) and prematurely degraded. Therefore, a compound which binds to and increases the stability of a mutant enzyme, may serve as a "chaperone" for the enzyme and increase the amount that can exit the ER and move to the lysosomes. In addition, because the folding and trafficking of some wild-type proteins is incomplete, with up to 70% of some wild-type proteins being degraded in some instances prior to reaching their final cellular location, the chaperones can be used to stabilize wild-type enzymes and increase the amount of enzyme which can exit the ER and be trafficked to lysosomes.

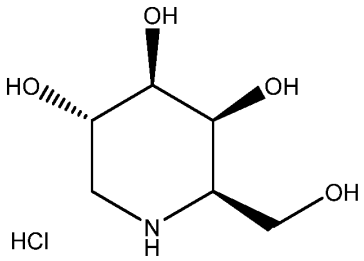
[00107] In one or more embodiments, the pharmacological chaperone comprises migalastat or a salt thereof. The compound migalastat, also known as 1-

deoxygalactonojirimycin (1-DGJ) or (2R,3S,4R,5S)-2-(hydroxymethyl) piperdine-3,4,5-triol is a compound having the following chemical formula:



Migalastat free base

[00108] As discussed herein, pharmaceutically acceptable salts of migalastat may also be used in the present invention. When a salt of migalastat is used, the dosage of the salt will be adjusted so that the dose of migalastat received by the patient is equivalent to the amount which would have been received had the migalastat free base been used. One example of a pharmaceutically acceptable salt of migalastat is migalastat HCl:



Migalastat HCl

[00109] Migalastat is a low molecular weight iminosugar and is an analogue of the terminal galactose of GL-3. *In vitro* and *in vivo* pharmacologic studies have demonstrated that migalastat acts as a pharmacological chaperone, selectively and reversibly binding, with high affinity, to the active site of wild-type α-Gal A and specific mutant forms of α-Gal A, the genotypes of which are referred to as HEK assay amenable mutations. Migalastat binding stabilizes these mutant forms of α-Gal A in the endoplasmic reticulum facilitating their proper trafficking to lysosomes where dissociation of migalastat allows α-Gal A to reduce the level of GL-3 and other substrates. Approximately 30-50% of patients with Fabry disease have HEK

assay amenable mutations; the majority of which are associated with the classic phenotype of the disease.

[00110] HEK assay amenable mutations include at least those mutations listed in a pharmacological reference table (*e.g.*, the ones recited in the U.S. or International Product labels for a migalastat product such as GALAFOLD®). As used herein, "pharmacological reference table" refers to any publicly accessible written or electronic record, included in either the product label within the packaging of a migalastat product (*e.g.*, GALAFOLD®) or in a website accessible by health care providers, that conveys whether a particular mutation or variant is responsive to migalastat (*e.g.*, GALAFOLD®) PC therapy, and is not necessarily limited to written records presented in tabular form. In one embodiment of the present invention, a "pharmacological reference table" thus refers to any depository of information that includes one or more amenable mutations or variants. An exemplary pharmacological reference table for HEK assay amenable mutations can be found in the summary of product characteristics and/or prescribing information for GALAFOLD® in various countries in which GALAFOLD® is approved for use, or at a website such as www.galafoldamenabilitytable.com or www.fabrygenevariantsearch.com, each of which is hereby incorporated by reference in its entirety.

[00111] An exemplary pharmacological reference table for HEK assay amenable mutations is provided in Table 1 below. In one or more embodiments, if a double mutation is present on the same chromosome (males and females), that patient is considered HEK assay amenable if the double mutation is present in one entry in Table 1 (*e.g.*, D55V/Q57L). In some embodiments, if a double mutation is present on different chromosomes (only in females) that patient is considered HEK assay amenable if either one of the individual mutations is present in Table 1.

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.7C>G	c.C7G	L3V
c.8T>C	c.T8C	L3P
c.[11G>T; 620A>C]	c.G11T/A620C	R4M/Y207S
c.37G>A	c.G37A	A13T
c.37G>C	c.G37C	A13P
c.43G>A	c.G43A	A15T
c.44C>G	c.C44G	A15G

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.53T>G	c.T53G	F18C
c.58G>C	c.G58C	A20P
c.59C>A	c.C59A	A20D
c.70T>C or c.70T>A	c.T70C or c.T70A	W24R
c.70T>G	c.T70G	W24G
c.72G>C or c.72G>T	c.G72C or c.G72T	W24C
c.95T>C	c.T95C	L32P
c.97G>C	c.G97C	D33H
c.97G>T	c.G97T	D33Y
c.98A>G	c.A98G	D33G
c.100A>G	c.A100G	N34D
c.101A>C	c.A101C	N34T
c.101A>G	c.A101G	N34S
c.102T>G or c.102T>A	c.T102G or c.T102A	N34K
c.103G>C or c.103G>A	c.G103C or c.G103A	G35R
c.104G>A	c.G104A	G35E
c.104G>C	c.G104C	G35A
c.104G>T	c.G104T	G35V
c.107T>C	c.T107C	L36S
c.107T>G	c.T107G	L36W
c.108G>C or c.108G>T	c.G108C or c.G108T	L36F
c.109G>A	c.G109A	A37T
c.110C>T	c.C110T	A37V
c.122C>T	c.C122T	T41I
c.124A>C or c.124A>T	c.A124C or c.A124T	M42L
c.124A>G	c.A124G	M42V
c.125T>A	c.T125A	M42K
c.125T>C	c.T125C	M42T
c.125T>G	c.T125G	M42R
c.126G>A or c.126G>C or c.126G>T	c.G126A or c.G126C or c.G126T	M42I
c.137A>C	c.A137C	H46P
c.142G>C	c.G142C	E48Q
c.152T>A	c.T152A	M51K
c.153G>A or c.153G>T or c.153G>C	c.G153A or c.G153T or c.G153C	M51I
c.157A>G	c.A157G	N53D
c.[157A>C; 158A>T]	c.A157C/A158T	N53L
c.160C>T	c.C160T	L54F
c.161T>C	c.T161C	L54P
c.164A>G	c.A164G	D55G
c.164A>T	c.A164T	D55V
c.[164A>T; 170A>T]	c.A164T/A170T	D55V/Q57L
c.167G>T	c.G167T	C56F
c.167G>A	c.G167A	C56Y

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.170A>T	c.A170T	Q57L
c.175G>A	c.G175A	E59K
c.178C>A	c.C178A	P60T
c.178C>T	c.C178T	P60S
c.179C>T	c.C179T	P60L
c.196G>A	c.G196A	E66K
c.197A>G	c.A197G	E66G
c.207C>A or c.207C>G	c.C207A or c.C207G	F69L
c.214A>G	c.A214G	M72V
c.216G>A or c.216G>T or c.216G>C	c.G216A or c.G216T or c.G216C	M72I
c.218C>T	c.C218T	A73V
c.227T>C	c.T227C	M76T
c.239G>A	c.G239A	G80D
c.247G>A	c.G247A	D83N
c.253G>A	c.G253A	G85S
c.254G>A	c.G254A	G85D
c.[253G>A; 254G>A]	c.G253A/G254A	G85N
c.[253G>A; 254G>T; 255T>G]	c.G253A/G254T/T255G	G85M
c.261G>C or c.261G>T	c.G261C or c.G261T	E87D
c.263A>C	c.A263C	Y88S
c.265C>T	c.C265T	L89F
c.272T>C	c.T272C	I91T
c.288G>A or c.288G>T or c.288G>C	c.G288A or c.G288T or c.G288C	M96I
c.289G>C	c.G289C	A97P
c.290C>T	c.C290T	A97V
c.305C>T	c.C305T	S102L
c.311G>T	c.G311T	G104V
c.316C>T	c.C316T	L106F
c.322G>A	c.G322A	A108T
c.326A>G	c.A326G	D109G
c.334C>G	c.C334G	R112G
c.335G>A	c.G335A	R112H
c.337T>A	c.T337A	F113I
c.337T>C or c.339T>A or c.339T>G	c.T337C or c.T339A or c.T339G	F113L
c.352C>T	c.C352T	R118C
c.361G>A	c.G361A	A121T
c.368A>G	c.A368G	Y123C
c.373C>T	c.C373T	H125Y
c.374A>T	c.A374T	H125L
c.376A>G	c.A376G	S126G
c.383G>A	c.G383A	G128E
c.399T>G	c.T399G	I133M

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.404C>T	c.C404T	A135V
c.408T>A or c.408T>G	c.T408A or c.T408G	D136E
c.416A>G	c.A416G	N139S
c.419A>C	c.A419C	K140T
c.427G>A	c.G427A	A143T
c.431G>A	c.G431A	G144D
c.431G>T	c.G431T	G144V
c.434T>C	c.T434C	F145S
c.436C>T	c.C436T	P146S
c.437C>G	c.C437G	P146R
c.454T>C	c.T454C	Y152H
c.455A>G	c.A455G	Y152C
c.466G>A	c.G466A	A156T
c.467C>T	c.C467T	A156V
c.471G>C or c.471G>T	c.G471C or c.G471T	Q157H
c.484T>G	c.T484G	W162G
c.493G>C	c.G493C	D165H
c.494A>G	c.A494G	D165G
c.[496C>G; 497T>G]	c.C496G/T497G	L166G
c.496C>G	c.C496G	L166V
c.496_497delinsTC	c.496_497delinsTC	L166S
c.499C>G	c.C499G	L167V
c.506T>C	c.T506C	F169S
c.511G>A	c.G511A	G171S
c.520T>C	c.T520C	C174R
c.520T>G	c.T520G	C174G
c.525C>G or c.525C>A	c.C525G or c.C525A	D175E
c.539T>G	c.T539G	L180W
c.540G>C	c.G540C	L180F
c.548G>C	c.G548C	G183A
c.548G>A	c.G548A	G183D
c.550T>A	c.T550A	Y184N
c.551A>G	c.A551G	Y184C
c.553A>G	c.A553G	K185E
c.559A>G	c.A559G	M187V
c.559_564dup	c.559_564dup	p.M187_S188dup
c.560T>C	c.T560C	M187T
c.561G>T or c.561G>A or c.561G>C	c.G561T or c.G561A or c.G561C	M187I
c.572T>A	c.T572A	L191Q
c.580A>G	c.A580G	T194A
c.581C>T	c.C581T	T194I
c.584G>T	c.G584T	G195V
c.586A>G	c.A586G	R196G
c.593T>C	c.T593C	I198T

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.595G>A	c.G595A	V199M
c.596T>C	c.T596C	V199A
c.596T>G	c.T596G	V199G
c.599A>G	c.A599G	Y200C
c.602C>T	c.C602T	S201F
c.602C>A	c.C602A	S201Y
c.608A>T	c.A608T	E203V
c.609G>C or c.609G>T	c.G609C or c.G609T	E203D
c.610T>G	c.T610G	W204G
c.613C>A	c.C613A	P205T
c.613C>T	c.C613T	P205S
c.614C>T	c.C614T	P205L
c.619T>C	c.T619C	Y207H
c.620A>C	c.A620C	Y207S
c.623T>G	c.T623G	M208R
c.628C>T	c.C628T	P210S
c.629C>T	c.C629T	P210L
c.638A>G	c.A638G	K213R
c.638A>T	c.A638T	K213M
c.640C>T	c.C640T	P214S
c.641C>T	c.C641T	P214L
c.643A>G	c.A643G	N215D
c.644A>G	c.A644G	N215S
c.644A>T	c.A644T	N215I
c.[644A>G; 937G>T]	c.A644G/G937T	N215S/D313Y
c.646T>G	c.T646G	Y216D
c.647A>C	c.A647C	Y216S
c.647A>G	c.A647G	Y216C
c.655A>C	c.A655C	I219L
c.656T>A	c.T656A	I219N
c.656T>C	c.T656C	I219T
c.659G>A	c.G659A	R220Q
c.659G>C	c.G659C	R220P
c.662A>C	c.A662C	Q221P
c.671A>C	c.A671C	N224T
c.671A>G	c.A671G	N224S
c.673C>G	c.C673G	H225D
c.683A>G	c.A683G	N228S
c.687T>A or c.687T>G	c.T687A or c.T687G	F229L
c.695T>C	c.T695C	I232T
c.713G>A	c.G713A	S238N
c.716T>C	c.T716C	I239T
c.720G>C or c.720G>T	c.G720C or c.G720T	K240N
c.724A>G	c.A724G	I242V
c.724A>T	c.A724T	I242F

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.725T>A	c.T725A	I242N
c.725T>C	c.T725C	I242T
c.728T>G	c.T728G	L243W
c.729G>C or c.729G>T	c.G729C or c.G729T	L243F
c.730G>A	c.G730A	D244N
c.730G>C	c.G730C	D244H
c.733T>G	c.T733G	W245G
c.740C>G	c.C740G	S247C
c.747C>G or c.747C>A	c.C747G or c.C747A	N249K
c.748C>A	c.C748A	Q250K
c.749A>C	c.A749C	Q250P
c.749A>G	c.A749G	Q250R
c.750G>C	c.G750C	Q250H
c.758T>C	c.T758C	I253T
c.758T>G	c.T758G	I253S
c.760-762delGTT	c.760_762delGTT	p.V254del
c.769G>C	c.G769C	A257P
c.770C>G	c.C770G	A257G
c.772G>C or c.772G>A	c.G772C or c.G772A	G258R
c.773G>T	c.G773T	G258V
c.776C>G	c.C776G	P259R
c.776C>T	c.C776T	P259L
c.779G>A	c.G779A	G260E
c.779G>C	c.G779C	G260A
c.781G>A	c.G781A	G261S
c.781G>C	c.G781C	G261R
c.781G>T	c.G781T	G261C
c.788A>G	c.A788G	N263S
c.790G>T	c.G790T	D264Y
c.794C>T	c.C794T	P265L
c.800T>C	c.T800C	M267T
c.805G>A	c.G805A	V269M
c.806T>C	c.T806C	V269A
c.809T>C	c.T809C	I270T
c.810T>G	c.T810G	I270M
c.811G>A	c.G811A	G271S
c.[811G>A; 937G>T]	c.G811A/G937T	G271S/D313Y
c.812G>A	c.G812A	G271D
c.823C>G	c.C823G	L275V
c.827G>A	c.G827A	S276N
c.829T>G	c.T829G	W277G
c.831G>T or c.831G>C	c.G831T or c.G831C	W277C
c.832A>T	c.A832T	N278Y
c.835C>G	c.C835G	Q279E
c.838C>A	c.C838A	Q280K

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.840A>T or c.840A>C	c.A840T or c.A840C	Q280H
c.844A>G	c.A844G	T282A
c.845C>T	c.C845T	T282I
c.850A>G	c.A850G	M284V
c.851T>C	c.T851C	M284T
c.860G>T	c.G860T	W287L
c.862G>C	c.G862C	A288P
c.866T>G	c.T866G	I289S
c.868A>C or c.868A>T	c.A868C or c.A868T	M290L
c.869T>C	c.T869C	M290T
c.870G>A or c.870G>C or c.870G>T	c.G870A or c.G870C or c.G870T	M290I
c.871G>A	c.G871A	A291T
c.877C>A	c.C877A	P293T
c.881T>C	c.T881C	L294S
c.884T>G	c.T884G	F295C
c.886A>G	c.A886G	M296V
c.886A>T or c.886A>C	c.A886T or c.A886C	M296L
c.887T>C	c.T887C	M296T
c.888G>A or c.888G>T or c.888G>C	c.G888A or c.G888T or c.G888C	M296I
c.893A>G	c.A893G	N298S
c.897C>G or c.897C>A	c.C897G or c.C897A	D299E
c.898C>T	c.C898T	L300F
c.899T>C	c.T899C	L300P
c.901C>G	c.C901G	R301G
c.902G>C	c.G902C	R301P
c.902G>A	c.G902A	R301Q
c.902G>T	c.G902T	R301L
c.907A>T	c.A907T	I303F
c.908T>A	c.T908A	I303N
c.911G>A	c.G911A	S304N
c.911G>C	c.G911C	S304T
c.919G>A	c.G919A	A307T
c.922A>G	c.A922G	K308E
c.924A>T or c.924A>C	c.A924T or c.A924C	K308N
c.925G>C	c.G925C	A309P
c.926C>T	c.C926T	A309V
c.928C>T	c.C928T	L310F
c.931C>G	c.C931G	L311V
c.935A>G	c.A935G	Q312R
c.936G>T or c.936G>C	c.G936T or c.G936C	Q312H
c.937G>T	c.G937T	D313Y
c.[937G>T; 1232G>A]	c.G937T/G1232A	D313Y/G411D
c.938A>G	c.A938G	D313G

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.946G>A	c.G946A	V316I
c.947T>G	c.T947G	V316G
c.950T>C	c.T950C	I317T
c.955A>T	c.A955T	I319F
c.956T>C	c.T956C	I319T
c.959A>T	c.A959T	N320I
c.962A>G	c.A962G	Q321R
c.962A>T	c.A962T	Q321L
c.963G>C or c.963G>T	c.G963C or c.G963T	Q321H
c.964G>A	c.G964A	D322N
c.964G>C	c.G964C	D322H
c.966C>A or c.966C>G	c.C966A or c.C966G	D322E
c.968C>G	c.C968G	P323R
c.973G>A	c.G973A	G325S
c.973G>C	c.G973C	G325R
c.978G>C or c.978G>T	c.G978C or c.G978T	K326N
c.979C>G	c.C979G	Q327E
c.980A>T	c.A980T	Q327L
c.983G>C	c.G983C	G328A
c.989A>G	c.A989G	Q330R
c.1001G>A	c.G1001A	G334E
c.1010T>C	c.T1010C	F337S
c.1012G>A	c.G1012A	E338K
c.1016T>A	c.T1016A	V339E
c.1027C>A	c.C1027A	P343T
c.1028C>T	c.C1028T	P343L
c.1033T>C	c.T1033C	S345P
c.1046G>C	c.G1046C	W349S
c.1055C>G	c.C1055G	A352G
c.1055C>T	c.C1055T	A352V
c.1061T>A	c.T1061A	I354K
c.1066C>G	c.C1066G	R356G
c.1066C>T	c.C1066T	R356W
c.1067G>A	c.G1067A	R356Q
c.1067G>C	c.G1067C	R356P
c.1072G>C	c.G1072C	E358Q
c.1073A>C	c.A1073C	E358A
c.1073A>G	c.A1073G	E358G
c.1074G>T or c.1074G>C	c.G1074T or c.G1074C	E358D
c.1076T>C	c.T1076C	I359T
c.1078G>A	c.G1078A	G360S
c.1078G>T	c.G1078T	G360C
c.1079G>A	c.G1079A	G360D
c.1082G>A	c.G1082A	G361E
c.1082G>C	c.G1082C	G361A

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.1084C>A	c.C1084A	P362T
c.1085C>T	c.C1085T	P362L
c.1087C>T	c.C1087T	R363C
c.1088G>A	c.G1088A	R363H
c.1102G>A	c.G1102A	A368T
c.1117G>A	c.G1117A	G373S
c.1124G>A	c.G1124A	G375E
c.1153A>G	c.A1153G	T385A
c.1168G>A	c.G1168A	V390M
c.1172A>C	c.A1172C	K391T
c.1175G>C	c.G1175C	R392T
c.1184G>A	c.G1184A	G395E
c.1184G>C	c.G1184C	G395A
c.1192G>A	c.G1192A	E398K
c.1202_1203insGACTTC	c.1202_1203insGACTTC	p.T400_S401dup
c.1208T>C	c.T1208C	L403S
c.1225C>G	c.C1225G	P409A
c.1225C>T	c.C1225T	P409S
c.1225C>A	c.C1225A	P409T
c.1228A>G	c.A1228G	T410A
c.1229C>T	c.C1229T	T410I
c.1232G>A	c.G1232A	G411D
c.1235C>A	c.C1235A	T412N
c.1253A>G	c.A1253G	E418G
c.1261A>G	c.A1261G	M421V

Dosing, Formulation and Administration

[00112] In one or more embodiments, the Fabry patient is administered migalastat or salt thereof at a frequency of once every other day (also referred to as "QOD"). In various embodiments, the doses described herein pertain to migalastat hydrochloride or an equivalent dose of migalastat or a salt thereof other than the hydrochloride salt. In some embodiments, these doses pertain to the free base of migalastat. In alternate embodiments, these doses pertain to a salt of migalastat. In further embodiments, the salt of migalastat is migalastat hydrochloride. The administration of migalastat or a salt of migalastat is referred to herein as "migalastat therapy".

[00113] The effective amount of migalastat or salt thereof can be in the range from about 100 mg FBE to about 150 mg FBE. Exemplary doses include about 100 mg FBE, about 105 mg FBE, about 110 mg FBE, about 115 mg FBE, about 120 mg FBE, about 123 mg FBE, about 125 mg FBE, about 130 mg FBE, about 135 mg FBE, about 140 mg FBE, about 145 mg FBE or about 150 mg FBE.

[00114] Again, it is noted that 150 mg of migalastat hydrochloride is equivalent to 123 mg of the free base form of migalastat. Thus, in one or more embodiments, the dose is 150 mg of migalastat hydrochloride or an equivalent dose of migalastat or a salt thereof other than the hydrochloride salt, administered at a frequency of once every other day. As set forth above, this dose is referred to as 123 mg FBE of migalastat. In further embodiments, the dose is 150 mg of migalastat hydrochloride administered at a frequency of once every other day. In other embodiments, the dose is 123 mg of the migalastat free base administered at a frequency of once every other day.

[00115] In various embodiments, the effective amount is about 122 mg, about 128 mg, about 134 mg, about 140 mg, about 146 mg, about 150 mg, about 152 mg, about 159 mg, about 165 mg, about 171 mg, about 177 mg or about 183 mg of migalastat hydrochloride.

[00116] Accordingly, in various embodiments, migalastat therapy includes administering 123 mg FBE at a frequency of once every other day, such as 150 mg of migalastat hydrochloride every other day.

[00117] The administration of migalastat or salt thereof may be for a certain period of time. In one or more embodiments, the migalastat or salt thereof is administered for a duration of at least 28 days, such as at least 30, 60 or 90 days or at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, 20, 24, 30 or 36 months or at least 1, 2, 3, 4 or 5 years. In various embodiments, the migalastat therapy is long-term migalastat therapy of at least 6 months, such as at least 6, 7, 8, 9, 10, 11, 12, 16, 20, 24, 30 or 36 months or at least 1, 2, 3, 4 or 5 years.

[00118] Administration of migalastat or salt thereof according to the present invention may be in a formulation suitable for any route of administration, but is preferably administered in an oral dosage form such as a tablet, capsule or solution. As one example, the patient is orally administered capsules each containing 150 mg migalastat hydrochloride or an equivalent dose of migalastat or a salt thereof other than the hydrochloride salt.

[00119] In some embodiments, the PC (*e.g.*, migalastat or salt thereof) is administered orally. In one or more embodiments, the PC (*e.g.*, migalastat or salt thereof) is administered by

injection. The PC may be accompanied by a pharmaceutically acceptable carrier, which may depend on the method of administration.

[00120] In one or more embodiments, the PC (*e.g.*, migalastat or salt thereof) is administered as monotherapy, and can be in a form suitable for any route of administration, including *e.g.*, orally in the form tablets or capsules or liquid, or in sterile aqueous solution for injection. In other embodiments, the PC is provided in a dry lyophilized powder to be added to the formulation of the replacement enzyme during or immediately after reconstitution to prevent enzyme aggregation *in vitro* prior to administration.

[00121] When the PC (*e.g.*, migalastat or salt thereof) is formulated for oral administration, the tablets or capsules can be prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulfate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or another suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p- hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated to give controlled release of the active chaperone compound.

[00122] The pharmaceutical formulations of the PC (*e.g.*, migalastat or salt thereof) suitable for parenteral/injectable use generally include sterile aqueous solutions (where water soluble), or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example,

water, ethanol, polyol (for example, glycerol, propylene glycol, and polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, benzyl alcohol, sorbic acid, and the like. In many cases, it will be reasonable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monosterate and gelatin.

[00123] Sterile injectable solutions are prepared by incorporating the purified enzyme (if any) and the PC (*e.g.*, migalastat or salt thereof) in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter or terminal sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

[00124] The formulation can contain an excipient. Pharmaceutically acceptable excipients which may be included in the formulation are buffers such as citrate buffer, phosphate buffer, acetate buffer, bicarbonate buffer, amino acids, urea, alcohols, ascorbic acid, and phospholipids; proteins, such as serum albumin, collagen, and gelatin; salts such as EDTA or EGTA, and sodium chloride; liposomes; polyvinylpyrollidone; sugars, such as dextran, mannitol, sorbitol, and glycerol; propylene glycol and polyethylene glycol (*e.g.*, PEG-4000, PEG-6000); glycerol; glycine or other amino acids; and lipids. Buffer systems for use with the formulations include citrate; acetate; bicarbonate; and phosphate buffers. Phosphate buffer is a preferred embodiment.

[00125] The route of administration of the chaperone compound may be oral or parenteral, including intravenous, subcutaneous, intra-arterial, intraperitoneal, ophthalmic, intramuscular, buccal, rectal, vaginal, intraorbital, intracerebral, intradermal, intracranial, intraspinal, intraventricular, intrathecal, intracisternal, intracapsular, intrapulmonary, intranasal, transmucosal, transdermal, or via inhalation.

[00126] Administration of the above-described parenteral formulations of the chaperone compound may be by periodic injections of a bolus of the preparation, or may be administered by intravenous or intraperitoneal administration from a reservoir which is external (*e.g.*, an i.v. bag) or internal (*e.g.*, a bioerodable implant).

[00127] Embodiments relating to pharmaceutical formulations and administration may be combined with any of the other embodiments of the invention, for example embodiments relating to methods of treating patients with Fabry disease, methods of treating ERT-naïve Fabry patients, methods of treating ERT-experienced Fabry patients, methods of enhancing cardiac function (*e.g.,* left ventricular systolic function), methods of stabilizing cardiac function (*e.g.,* left ventricular systolic function), methods of increasing MWFS, methods of stabilizing MWFS, methods of normalizing MWFS, methods of enhancing α-Gal A in a patient diagnosed with or suspected of having Fabry disease, use of a pharmacological chaperone for α-Gal A for the manufacture of a medicament for treating a patient diagnosed with Fabry disease or to a pharmacological chaperone for α-Gal A for use in treating a patient diagnosed with Fabry disease as well as embodiments relating to amenable mutations, the PCs and suitable dosages thereof.

[00128] In one or more embodiments, the PC (*e.g.*, migalastat or salt thereof) is administered in combination with ERT. ERT increases the amount of protein by exogenously introducing wild-type or biologically functional enzyme by way of infusion. This therapy has been developed for many genetic disorders, including LSDs such as Fabry disease, as referenced above. After the infusion, the exogenous enzyme is expected to be taken up by tissues through non-specific or receptor-specific mechanism. In general, the uptake efficiency is not high, and the circulation time of the exogenous protein is short. In addition, the exogenous protein is unstable and subject to rapid intracellular degradation as well as having the potential for adverse immunological reactions with subsequent treatments. In one or more embodiments, the chaperone is administered at the same time as replacement enzyme (*e.g.*, replacement α-Gal A). In some embodiments, the chaperone is co-formulated with the replacement enzyme (*e.g.*, replacement α-Gal A).

[00129] In one or more embodiments, a patient is switched from ERT to migalastat therapy. In some embodiments, a patient on ERT is identified, the patient's ERT is discontinued, and the patient begins receiving migalastat therapy. The migalastat therapy can be in accordance with any of the methods described herein.

Cardiac Function

[00130] The dosing regimens described herein can stabilize and/or enhance cardiac function (*e.g.,* left ventricular systolic function) in Fabry patients. As untreated Fabry patients typically exhibit a deterioration of cardiac function over time, both enhancements in and maintenance of cardiac function are indications of a benefit of migalastat therapy. As described in further detail in the Examples below, Phase 3 studies have found that migalastat therapy increases and/or stabilizes MWFS in both ERT-experienced and ERT-naïve patients. These Phase 3 studies also found that migalastat therapy normalizes MWFS in some patients with impaired MWFS. Accordingly, migalastat therapy can be used to treat Fabry patients by stabilizing MWFS, increasing MWFS and/or normalizing MWFS in ERT-naïve and/or ERT-experienced Fabry patients, including patients with impaired MWFS.

[00131] The migalastat therapy may arrest or decrease the reduction in MWFS and/or increase MWFS for a Fabry patient compared to the same patient without treatment with migalastat therapy. In one or more embodiments, the migalastat therapy provides a change in MWFS for a patient that is greater than (i.e., more positive than) -2%, such as greater than or equal to about -1.5%, -1.4%, -1.3%, -1.2%, -1.1%, -1%, -0.9%, -08.%, -0.7%, -0.6%, -0.5%, -0.4%, -0.3%, -0.2%, -0.1%, 0%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 1.1%, 1.2%, 1.3%, 1.4%, 1.5%, 1.6%, 1.7%, 1.8%, 1.9%, 2%, 2.1%, 2.2%, 2.3%, 2.4% or 2.5%. In one or more embodiments, the Fabry patient is an ERT-experienced patient. In one or more embodiments, the Fabry patient is an ERT-naïve patient. In one or more embodiments, the Fabry patient has impaired MWFS prior to initiating migalastat therapy.

[00132] In one or more embodiments, the migalastat therapy provides an average change of MWFS in a group of ERT-naïve patients of at least about 0% after 12 months of administration of migalastat or a salt thereof. In various embodiments, the average increase in the group of ERT-naïve patients after 12 months of administration of migalastat or a salt thereof is at least about 0.05%, about 0.1%, about 0.15% or about 0.2%. In one or more embodiments, the ERT-naïve patients have impaired MWFS prior to initiating migalastat therapy.

[00133] In one or more embodiments, the migalastat therapy provides an average change of MWFS in a group of ERT-naïve patients of at least about 0% after 24 months of administration of migalastat or a salt thereof. In various embodiments, the average increase in

the group of ERT-naïve patients after 12 months of administration of migalastat or a salt thereof is at least about 0.05%, about 0.1%, about 0.15%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.1%, about 1.2%, about 1.3%, about 1.4% or about 1.5%. In one or more embodiments, the ERT-naïve patients have impaired MWFS prior to initiating migalastat therapy.

[00134] In one or more embodiments, the migalastat therapy provides an average change of MWFS in a group of ERT-naïve patients of at least about 0% after 36 months of administration of migalastat or a salt thereof. In various embodiments, the average increase in the group of ERT-naïve patients after 12 months of administration of migalastat or a salt thereof is at least about 0.05%, about 0.1%, about 0.15%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.1%, about 1.2%, about 1.3%, about 1.4% or about 1.5%. In one or more embodiments, the ERT-naïve patients have impaired MWFS prior to initiating migalastat therapy.

[00135] In one or more embodiments, the migalastat therapy provides an average change of MWFS in a group of ERT-naïve patients of at least about 0% after 48 months of administration of migalastat or a salt thereof. In various embodiments, the average increase in the group of ERT-naïve patients after 12 months of administration of migalastat or a salt thereof is at least about 0.05%, about 0.1%, about 0.15%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9% about 2%, about 2.1%, about 2.2%, about 2.3%, about 2.4% or about 2.5%. In one or more embodiments, the ERT-naïve patients have impaired MWFS prior to initiating migalastat therapy.

[00136] In one or more embodiments, the migalastat therapy provides an average change of MWFS in a group of ERT-naïve patients of greater about -1.5% after 30 months of administration of migalastat or a salt thereof. In various embodiments, the average increase in the group of ERT-naïve patients after 12 months of administration of migalastat or a salt thereof is greater than -1.5%, -1.4%, -1.3%, -1.2%, -1.1%, -1%, -0.9%, -08.%, -0.7%, -0.6%, -0.5%, -0.4%, -0.3%, -0.2%, -0.1% or 0%. In one or more embodiments, the ERT-experienced patients have impaired MWFS prior to initiating migalastat therapy.

EXAMPLES

EXAMPLE 1: Dosing Regimens for the Treatment of ERT-Naïve Fabry Patients Using Migalastat Hydrochloride

[00137] This example describes a Phase 3 study of migalastat therapy in ERT-naïve Fabry patients.

[00138] ***Patient Enrollment.*** Eligible patients were 16-74 years old and had genetically-confirmed Fabry disease; had either never received or had not received ERT for ≥6 months; had a GLA mutation that resulted in a mutant protein that would respond to migalastat, based on the human embryonic kidney-293 (HEK) assay used at the time of enrollment; had an eGFR >30 ml/minute/1.73m^2, and had a urinary GL-3 ≥4 times the upper limit of normal.

[00139] ***Study Design.*** Following eligibility-baseline assessments (2 months), patients were randomized to Stage 1-- 6 months of double-blind administration of 150 mg migalastat hydrochloride or placebo every other day. All patients completing Stage 1 were eligible to receive open-label migalastat in Stage 2 (months 6-12) and for an additional year (months 13-24) thereafter. The primary objective was to compare the effect of migalastat to placebo on kidney GL-3 as assessed by histological scoring of the number of inclusions in interstitial capillaries after 6 months of treatment. The secondary objectives of Stage 1 were to compare the effect of migalastat to placebo on urine GL-3 levels, on renal function, 24-hours urinary protein, and on safety and tolerability. The tertiary objectives were cardiac function, patient-reported outcomes, exploratory kidney analyses, and white blood cell α-Gal A activity. Study completers were eligible to enroll in the open-label extension study for up to 5 years.

[00140] ***Kidney Histology Assessment.*** Each patient underwent a baseline kidney biopsy, as well as repeat kidney biopsies at 6 and 12 months. The number of GL-3 inclusions per kidney interstitial capillary per patient at baseline, and at 6 and 12 months was quantitatively assessed in 300 capillaries by 3 independent pathologists blinded to treatment and visit. All values for each individual biopsy at a given time were averaged prior to statistical analysis.

[00141] GL-3 changes in podocytes, endothelial cells, and mesangial cells, and glomerular sclerosis, were assessed qualitatively by the same 3 pathologists blinded to treatment/visit.

[00142] ***Globotriaosylceramide and Globotriaosylsphingosine.*** Plasma lyso-Gb3 and 24-hour urine GL-3 were analyzed by liquid chromatography-mass-spectroscopy using a novel

stable isotope-labeled internal standard, 13C6-lyso-Gb3 (lower-limit-of-quantification: 0.200 ng/mL, 0.254 nmol/L).

[00143] ***Renal Function Assessment.*** Annualized rates of change (mL/min/1.73m^2/year) were calculated using Chronic Kidney Disease Epidemiology Collaboration–eGFR$_{CKD-EPI}$) and measured iohexol clearance–mGFR$_{iohexol}$).

[00144] ***Echocardiography.*** LVMi, left posterior wall thickness, diastolic, interventricular septum thickness, diastolic and other parameters were assessed through blinded, centralized evaluation.

[00145] ***Patient-Reported Outcomes.*** Patient-reported outcomes were assessed using the Gastrointestinal-Symptoms-Rating-Scale (GSRS), Short Form-36v2TM and Brief-Pain-Inventory-Pain-Severity-Component.

[00146] ***Safety Analysis and Adverse Events.*** Randomized patients receiving ≥1 dose were included in the safety analysis, which comprised vital signs, physical exams, electrocardiograms, clinical labs, and adverse events.

[00147] ***Statistical Analyses for Kidney Interstitial Capillary GL-3 Substrate.*** The primary Stage 1 (6 month) endpoint (ITT population with baseline biopsies, n=64) was the proportion of patients in the migalastat and placebo groups with a ≥50% reduction in GL-3 inclusions per interstitial capillary. Two other Stage 1 endpoints were assessed (modified-ITT population: randomized patients with paired baseline and month 6 biopsies; n=60): percent change in GL-3 inclusions per interstitial capillary, and percent of interstitial capillaries with zero GL-3 inclusions.

[00148] Efficacy analyses for GL-3 inclusions per interstitial capillary and other pre-specified endpoints in Stage 2 (months 6-12) and the open-label-extension (months 12-24) were based on the modified intention to treat (mITT) – population consisting of randomized patients with mutant α-Gal A enzyme shown to be suitable for migalastat treatment by the validated assay; n=50).

Results

[00149] **Baseline Characteristics.** Sixty-seven patients (16-74 years-old; 64% female) with potentially responsive mutant α-Gal A were randomized (ITT population). Table 2 provides the baseline characteristics for the 50 patients in the ITT population with suitable mutant α-Gal A. There were no statistically significant differences in baseline parameters.

Table 2: Baseline Characteristics

Parameter	Treatment Group		Total (N=50)
	Migalastat HCl (N=28)	Placebo to Migalastat HCl (N=22)	
Age (year) (n)	28	22	50
Mean±SD	41.5±13	45.1±8.0	43.1±11
Median	37.0	45.5	45.0
Weight (kg) (n)	28	22	50
Mean±SD	72.6±15.35	76.1±16.52	74.1±15.81
Median	72.3	74.0	72.8
Number of Years of Diagnosis of Fabry Disease (n)	28	21	49
Mean±SD	5.6±6.89	7.3±8.80	6.3±7.73
Median	4.1	4.1	4.1
Number of patients previously on ERT (>6 months prior to baseline) (%)	4 (14.3%)	7 (31.8%)	11 (22.0%)
Use of ACEi/ARB/Ri at Baseline			
Yes (%)	9 (32.1%)	12 (54.5%)	21 (42.0%)
No (%)	19 (67.9%)	10 (45.5%)	29 (58.0%)
Proteinuria >150mg/24h (%)	17 (60.7%)	18 (81.8%)	35 (70.0%)
Proteinuria >300mg/24h (%)	8 (28.6%)	11 (50.0%)	19 (38.0%)
Proteinuria >1000mg/24h (%)	3 (10.7%)	3 (13.6%)	6 (12.0%)
mGFR $_{Iohexol}$ (mL/min/1.73m^2) (n)	27	21	48
Mean±SD	79.95±30.9	83.12±22.8	81.34±27.5
Median	84.90	82.20	83.40
eGFR$_{CKD-EPI}$ (mL/min/1.73m^2)	28	22	50
Mean±SD	94.4±27.0	90.6±17.1	92.7±23.0
Median	96.6	93.5	94.0
Lyso-Gb$_3$ (n)	18	13	31
Mean (nmol/L) ±SD	47.3±62	41.9±39	45.0±53

[00150] Published reports of clinical phenotype(s) associated with the genotypes of patients with suitable mutations (n=50) indicate that 30 (60%) had mutations associated with the classic phenotype of Fabry disease, one (2%) with the non-classic phenotype, three (6%) with both phenotypes, and 16 (32%) not yet classified. Residual WBC α-Gal A activity <3% was found in 14 of 16 (87%) males; 29 of 31 (94%) males and females had elevated plasma lyso-Gb3, and 47 of 50 (94%) males and females had multi-organ system disease.

[00151] ***Baseline MWFS.*** At baseline, impaired MWFS (<15% for females and <14% for males) was reported in 9 patients.

[00152] **Migalastat and Cardiac Function.** This study of ERT-naïve patients found that migalastat therapy increased MWFS in patients with impaired MWFS at baseline. Table 3 below shows the change from baseline in MWFS after migalastat therapy.

Table 3: Percent Changes From Baseline in MWFS Over Time with Migalastat Therapy in Patients With Impaired MWFS at Baseline (Patients with Amenable Mutations)

	Baseline Mean MWFS = 11.3% **Timepoint**				
	Month 12	**Month 24**	**Month 36**	**Month 48**	**LOCF**
n	7	8	4	3	8
Mean change from baseline	0.1%	1.4%	1.4%	2.4%	1.9%
95% CI	–1.2%, –1.4%	–1.3%, 4.0%	–1.5%, 4.3%	–2.1%, 6.9%	–0.8%, 4.5%
Any increase	2/7 (29%)	5/8 (63%)	3/4 (75%)	3/3 (100%)	6/8 (75%)
Normalization	0	2/8 (25%)	2/4 (50%)	2/3 (67%)	3/8 (38%)

Last observation carried forward (LOCF) analyses are based on last study assessment including any unscheduled or early termination visits; Abnormal MWFS is <15% for females and <14% for males.

[00153] As can be seen from Table 3, LOCF analysis of ERT-naïve patients with impaired MWFS at baseline showed mean changes in MWFS of 1.9% (95% CI –0.8%, 4.5%;

n=8) over 48 months of migalastat therapy. 6/8 (75%) of patients had an increase of MWFS after migalastat therapy, with 3/8 (38%) demonstrating normalization of MWFS.

[00154] MWFS was also analyzed in patients with LVH at baseline. The change from baseline in MWFS in patients with LVH at baseline is provided in Table 4 below:

Table 4: Change From Baseline in MWFS With Migalastat in Patients With LVH at Baseline (Patients with Amenable Mutations)

	Baseline	Change from Baseline				
		Month 12	Month 24	Month 36	Month 48	LOCF
n	10	9	9	5	4	10
%, mean (SD) or (95% CI)	12.2 (2.6)	0.2 (–0.8, 1.1)	0.9 (–1.6, 3.4)	0.7 (–2.1, 3.4)	0.6 (–5.7, 6.9)	1.0 (–1.5, 3.5)
Any increase	–	4/9 (44%)	5/9 (56%)	3/5 (60%)	3/4 (75%)	7/10 (70%)
Normalization	–	2/9 (22%)	2/9 (22%)	1/5 (20%)	0	2/10 (20%)

LOCF analyses are based on last study assessment including any unscheduled or early termination visits; LVH subgroup: LVMi >95 g/m2 (females) or >115 g/m2 (males).

[00155] As can be seen from Table 4, LOCF analysis of ERT-naïve patients with LVH at baseline showed mean changes in MWFS of 1.0% (95% CI –1.5%, 3.5%; n=10) over 48 months of migalastat therapy. 7/10 (70%) of patients had an increase of MWFS after migalastat therapy, with 2/10 (20%) demonstrating normalization of MWFS.

[00156] **Safety and Adverse Events.** During Stage 1, the treatment-emergent adverse events were similar between groups. Adverse events with a higher frequency in patients receiving migalastat compared to placebo were headache (12/34 patients-35% versus 7/33 patients-21%) and nasopharyngitis (6/34 patients-18% versus 2/34–6%). The most frequently reported adverse events for Stage 2 were headache (9/63 patients-14%) and procedural pain (7/63 patients-11%–related to kidney biopsies) and, for the open-label-extension, proteinuria (9/57 patients-16%), headache (6/57 patients-11%), and bronchitis (6/57 patients-11%). Most

adverse events were mild or moderate in severity. No adverse events led to migalastat discontinuation.

[00157] Six patients experienced serious adverse events during Stage 1 (2: migalastat; 4: placebo), 5 during Stage 2, and 11 during the open-label-extension. Two serious adverse events were assessed as possibly related to migalastat by the investigator-- fatigue and paresthesia. Both occurred in the same patient between months 12-24 and resolved. No individual serious adverse event was reported by >1 patient. Two patients discontinued migalastat due to serious adverse events; both were deemed unrelated to migalastat. No deaths were reported.

[00158] Treatment-emergent proteinuria was reported in 9 patients (16%) between months 12-24, and in one case, was judged as migalastat-related. In 5 patients, the 24-month values were in the same range as baseline. Three patients with suitable mutations had overt baseline proteinuria (>1g/24-hr), which increased over 24 months. In 23/28 patients with baseline proteinuria <300 mg/24-h, 24-hour urine protein remained stable during migalastat treatment.

[00159] There was no progression to end-stage renal disease, no cardiac death and no stroke as defined in Banikazemi et al.. There was a single case of transient ischemic attack– judged unrelated to migalastat.

[00160] Analyses of vital sign, physical findings, laboratory, and ECG parameters did not reveal any clinically relevant effect of migalastat.

EXAMPLE 2: Dosing Regimens for the Treatment of ERT-Experienced Fabry Patients Using Migalastat Hydrochloride

[00161] This example describes a Phase 3 study of migalastat therapy in ERT-experienced Fabry patients.

[00162] ***Patient Enrollment.*** Eligible patients were 16-74 years old and had genetically-confirmed Fabry disease; had received ERT for ≥12 months; had a GLA mutation that resulted in a mutant protein that would respond to migalastat, based on the human embryonic kidney-293 (HEK) assay used at the time of enrollment; had an eGFR ≥ 30 ml/minute/1.73m^2; and had an ERT dose level and regimen that had been stable for at least 3 months.

[00163] ***Study Design.*** Following eligibility-baseline assessments, 57 patients were randomized to 18 months of migalastat therapy or ERT, followed by followed by 12 months of migalastat therapy. The migalastat dosing regimen was 150 mg of migalastat hydrochloride

every other day. The primary objective was to compare the effect of migalastat to ERT on renal function assessed by $mGFR_{iohexol}$ after 18 months of treatment. The secondary objectives were to compare the effect of migalastat to ERT on: renal function (assessed by eGFR and 24-hour urine protein); composite clinical outcome (assessed by time to occurrence of renal, cardiac, cerebrovascular events or death); cardiac function (assessed by echocardiography) and patient reported outcomes (pain and quality of life).

[00164] ***Baseline MWFS.*** At baseline, impaired MWFS (<15% for females and <14% for males) was reported in 19 (14 migalastat, 5 ERT) patients.

Results

[00165] **Migalastat and Cardiac Function.** This study of ERT-experienced patients found that migalastat therapy stabilized MWFS in patients with impaired MWFS at baseline. LOCF analysis of patients with impaired MWFS at baseline showed mean changes in baseline of –0.2% (95% CI –1.3%, 1.0%; n=14) over 30 months of migalastat therapy. LOCF analysis of patients with impaired MWFS at baseline showed mean changes in MWFS of -0.6% (95% CI –2.6%, 1.4%; n=5) over 18 months of treatment with ERT.

[00166] The embodiments described herein are intended to be illustrative of the present compositions and methods and are not intended to limit the scope of the present invention. Various modifications and changes consistent with the description as a whole and which are readily apparent to the person of skill in the art are intended to be included. The appended claims should not be limited by the specific embodiments set forth in the examples, but should be given the broadest interpretation consistent with the description as a whole.

[00167] Patents, patent applications, publications, product descriptions, GenBank Accession Numbers, and protocols are cited throughout this application, the disclosures of which are incorporated herein by reference in their entireties for all purposes.